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Leading Edge
Essay

Peering into the Crystal Ball: Influenza Pandemics and Vaccine Efficacy

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The looming threat of a new influenza virus pandemic has fueled ambitious efforts to devise more predictive parameters for assessing the risks associated with emergent virus strains. At the same time, a comprehensive understanding of critical factors that can accurately predict the outcome of vaccination is sorely needed in order to improve the effectiveness of influenza virus vaccines. Will new studies aimed at identifying adaptations required for virus transmissibility and systems-level analyses of influenza virus vaccine responses provide an improved framework for predictive models of viral adaptation and vaccine efficacy?

Introduction

The development of effective vaccines has altered the course of modern civilization by alleviating the scourges of humankind's most devastating pathogens. Illnesses caused by variola (smallpox), *Corynebacterium diphtheriae* (diphtheria), *Clostridium tetani* (tetanus), yellow fever virus (yellow fever), *Bordetella pertussis* (whooping cough), polio virus (polio), and measles virus (measles) have become as foreign to our youngest generations as telegrams and typewriters. Indeed, the success of vaccines to date is truly remarkable when considered in light of the rudimentary principles that guided their historical design (Stern and Markel, 2005). Ironically though, triumphs in the modern era of "rationale vaccine design" have been few and far between. Pathogens such as *Mycobacterium tuberculosis* (tuberculosis), *Plasmodium* spp. (causative agents of malaria), human immunodeficiency virus (HIV), and influenza A virus (IAV) continue to elude broad and highly efficacious vaccine-mediated protection, exerting devastating human and economic tolls. The factors that have limited the successful design of vaccines against these pathogens are complex. However, two prominent barriers stand out: (1) highly mutable/adaptable pathogens such as HIV and IAV evolve under immunological pressure to evade the pre-existing immunity afforded by vaccines. This has necessitated painstaking efforts to identify and target conserved epitopes of these viruses (Julien et al., 2012). (2) There is an astonishing paucity of robust, predictive immunological markers of vaccine efficacy. This, in turn, has precluded a comprehensive, mechanistic understanding of what differentiates successful vaccines from those that fail. The recognition of these challenges has ignited ambitious efforts to predict more accurately the behavior of the aforementioned pathogens and the vaccines designed to protect against them. Here, we review recent advances within the field of influenza virus research that are attempting to provide a more predictive basis for assessing the consequences of viral adaptations and the efficacy of vaccines, and we highlight the scientific and regulatory boundaries that must be overcome to achieve these goals. Two new studies focused on these important topics appear in this issue of *Cell*. Linster, van Boheemen, and colleagues (Linster et al., 2014) define a minimal set of mutations (and their associated phenotypes) that confer H5N1 viruses with the propensity to transmit in ferrets, and Tsang and colleagues (Tsang et al., 2014) use a systems biology approach to identify baseline immunological predictors of vaccine responses.

"Follow the Leader"

The most challenging issue facing IAV vaccinologists has always been the necessity to predict the antigenic characteristics of vaccine strains months in advance of the actual influenza season in order to allow sufficient time for vaccine production and distribution. This can be equated to a game of virological "follow the leader," wherein the medical and scientific communities are constantly chasing the unpredictable evolutionary trajectory of the virus. As a consequence of this guesswork, antigenic mismatch between strains included in the vaccine and the strains that eventually circulate is a regular occurrence. This can severely limit the effectiveness of a given vaccine. Of equal concern though is the suboptimal vaccine efficacy reported even during seasons in which near-perfect matches are achieved. Adding to this complexity are the vastly different qualities of responses elicited by the available influenza vaccine formulations, most notably those observed for inactivated, split vaccines in comparison to live attenuated vaccines (Osterholm et al., 2012). Recognition of the limitations that plague our current seasonal influenza virus vaccine approaches has catalyzed renewed efforts to understand and identify factors that may more accurately predict vaccine responses. Nevertheless, all of these issues pale in comparison to

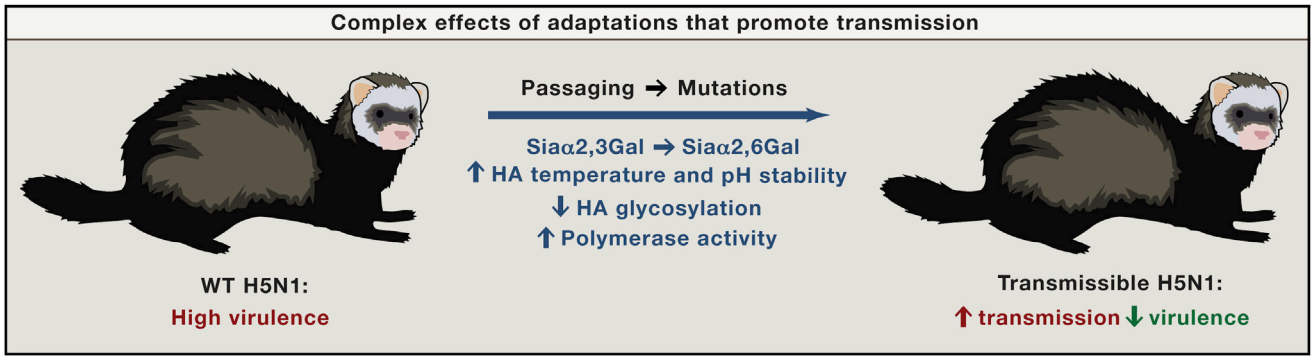

Figure 1. Predictive Factors of Influenza Virus Transmission

A number of adaptations (shown in blue) in H5N1 IAV were observed to confer the ability to transmit from ferret to ferret. This gain of function in transmission was accompanied by a loss of function in virulence. Predicting the pandemic risk associated with future outbreaks caused by novel IAV strains in humans will require a comprehensive understanding of which strains are capable of zoonotic transmission, the adaptations required for sustained human-to-human transmission, and the consequences of those adaptations on properties such as virulence and fitness. Siaα2,3Gal and Siaα2,6Gal = α 2,3-linked sialic acids and α 2,6-linked sialic acids, respectively.

the ever-present threat of a new and unexpected IAV pandemic. This has led to a redoubling of efforts to both detect and assess the risks associated with isolated epidemics caused by "exotic" IAV strains.

Predicting Pandemics?

Emergence of the "swine-origin" H1N1 IAV strain in 2009 that swept through Mexico and went on to cause the first pandemic of the 21st century served as a direct example of the inherent limitations precluding the accurate prediction of IAV dynamics (Girard et al., 2010). Never before had an IAV pandemic been caused by a virus carrying the same hemagglutinin (HA) and neuraminidase (NA) subtypes as one of the circulating seasonal strains. This served as an impetus to not only bolster surveillance efforts, but to also more thoroughly understand viral molecular determinants of virulence and transmission.

Despite the fact that at least 18 subtypes of IAV hemagglutinin (HA, H) and 11 subtypes of neuraminidase (NA, N) have so far been identified (Tong et al., 2013), only IAVs carrying H1, H2, or H3 and N1 or N2 have proven capable of sustaining transmission among humans and of causing pandemics. However, isolated outbreaks caused by other subtypes of IAV occur sporadically, igniting fears that a new pandemic may arise. Notable modern examples of such outbreaks have been those caused by H5N1 IAV in (primarly) Southeast Asia and a recent flurry of human cases caused by H7N9 IAV in China. Both of these viruses are of avian origin and are mainly transmitted to humans through exposure to high titers of virus from infected poultry (To et al., 2013).

The recent threat of a potentially devastating H5N1 pandemic prompted the NIH to fund a series of proposals aimed at identifying viral molecular determinants that might indicate adaptations that would confer the ability to transmit efficiently between mammals. This work, carried out by the groups of Yoshiro Kawaoka at The University of Wisconsin, Madison/University of Tokyo, Japan and Ron Fouchier at Erasmus University Rotterdam was first published in the journals *Nature* and *Science* in May and June of 2012, respectively (Herfst et al., 2012; Imai et al., 2012). The Kawaoka group used an unbiased genetic approach to generate a library of viruses containing mutations in the globular head domain of the HA from the A/Vietnam/1203/2004 H5N1 isolate in a nonpathogenic, mouse-adapted IAV background (PR8). Despite having been derived from a virus isolated from a human, this HA maintains an avian-like preference for binding to α2,3-linked sialic acids (Siaα2,3Gal), whereas IAVs that circulate in humans typically exhibit a preference for α2,6-linked sialic acids (Siaα2,6Gal). The authors identified a virus containing HA mutations E119G/V152I/N224K/Q226L that exhibited a receptor-binding profile resembling that of a seasonal human IAV isolate. Further analysis demonstrated that the N224K/Q226L mutations alone were primarily responsible for the switch from Siaα2,3Gal to Siaα2,6Gal binding (Imai et al., 2012).

The H5 HA mutations that conferred altered receptor binding characteristics were then rescued in the pandemic A/California/04/2009 (Cal/09) H1N1 virus background to assess how a recombinant of an avian H5N1 virus with the circulating Cal/09 H1N1 virus might behave. This strategy closely resembles previous studies performed by the Perez lab in the context of H9N2 IAV (Kimble et al., 2011). Despite causing a switch to human-like receptor binding, both E119G/V152I/N224K/Q226L and N224K/Q226L mutations tended to attenuate virus replication in the respiratory tract of ferrets. However, a secondary N158D or N158K mutation (which abolishes a glycosylation site at position 158) that appeared in animals inoculated with the N224K/Q226L variant improved virus replication in the upper respiratory tract. The combination of N158D/N224K/Q226L mutations conferred the ability to transmit between ferrets via respiratory droplets. A fourth mutation, which occurred naturally during the transmission experiment (T318I), enhanced the stability of the HA molecule and further enhanced transmission (Imai et al., 2012).

The Fouchier group used a similar but more classical virological approach to force adaptation of the A/Indonesia/5/2005 strain of H5N1 for the mammalian

respiratory tract. Both wild-type A/Indonesia/5/2005 and a ''pre-adapted'' recombinant virus containing a series of introduced mutations (HA Q222L/G224S is involved with switching receptor binding preference from Siaα2,3Gal to Siaα2,6Gal; PB2 E627K is related to a temperature adaptation that facilitates replication in the human upper respiratory tract) were serially passaged ten times in ferrets. Replication of the pre-adapted virus improved with passaging, while replication of the wild-type virus remained unchanged. The only mutation common among the wild-type and pre-adapted viruses after passaging was HA T156A. Strikingly, this mutation, like the N158D/K mutation observed by the Kawaoka group, abolished a putative N-linked glycosylation site. The passage 10 virus populations derived from animals inoculated with the pre-adapted virus were also capable of airborne transmission between ferrets, whereas the wild-type virus was not. All animals from which transmitted virus was recovered maintained the mutations introduced during pre-adaptation and also consistently harbored two novel amino acid substitutions in HA, H103Y (located at the trimer interface) and T156A (located proximal to the receptor binding site). However, the lowest number of substitutions relative to wild-type found in an isolated transmissible virus was nine (PB2-E627K, PB1-H99K, PB1-I368V, HA-H103Y, HA-T156A, HA-Q222L, HA-G224S, NP-R99K, and NP-S345N), which led to uncertainty regarding the minimal set of substitutions necessary to confer transmissibility (Herfst et al., 2012).

In this issue of *Cell*, Linster, van Boheemen, et al. expand upon this earlier work by elucidating the minimal set of substitutions required for airborne transmission of A/Indonesia/5/2005 H5N1 among ferrets and by describing the phenotypes associated with each change. In addition to the PB2-E627K, HA-Q222L/G224S, and HA-T156A substitutions described above, the authors found that HA-H103Y (stabilized HA with respect to high temperature and low pH) and PB1-H99Y (increased polymerase activity) constituted the minimal set of mutations required to transmit virus among ferrets (Linster et al., 2014). Critically, this type of analysis provides a more complete picture of the phenotypic properties of changes that may constitute increased risk of H5N1 transmissibility (Figure 1).

Taken together, these results suggested that a re-assortant virus carrying the HA of A/Vietnam/1203/2004 H5N1 and the remaining segments of A/California/04/2009 H1N1 would require as few as four amino acid substitutions in the HA molecule to become transmissible in mammals, whereas wild-type A/Indonesia/5/2005 H5N1 might require only five mutations (three in HA, one in PB1, and one in PB2) (Herfst et al., 2012; Imai et al., 2012). A meta-analysis of available H5N1 surveillance data revealed that, although many of the substitutions found to confer transmissibility among ferrets are rare in nature, HA substitutions N154D and T156A, which destroy an N-linked glycosylation site, and the PB2 E627K temperature adaptation are much more common and regularly occur together. Various mathematical models were developed to predict the probability that these substitutions would arise together during a natural infection and concluded that such a scenario was indeed probable. However, the proportion of viruses carrying the transmissible genotype as a function of the total virus population within a single host was extremely low, which would likely present a formidable barrier to efficient transmission (Russell et al., 2012).

Though these studies have undoubtedly enhanced our understanding of IAV transmissibility, their interpretability is constrained by several scientific and regulatory boundaries. Let us focus first on some crucial scientific considerations. The process of viral adaptation (particularly via serial passaging in a new host) has historically been exploited to achieve attenuation of strains to be used for vaccine formulation. This concept relies on the biological principle that the adaptation of new traits (i.e., transmissibility) is frequently accompanied by a loss of other traits for which there is less selective pressure (i.e., host range and/or virulence). Indeed, the Fouchier group observed that, although ferrets succumbed to intranasal inoculation with wild-type H5N1 at a dose of 1×10^6 TCID, the majority of the animals infected with the transmissible virus survived (Herfst et al., 2012). This clearly demonstrates that the gain of function (GOF) with regard to transmissibility was accompanied by a loss of function with regard to virulence. Thus, assessment of how adaptations in ferrets affect viral fitness, virulence, and transmission (both in birds and other mammalian species) is sorely needed to gain a truly holistic perspective of the likelihood that these viruses might cause a pandemic and what characteristics such a pandemic might exhibit. Indeed, studies in mice (Zaraket et al., 2013) have clearly demonstrated that the H5N1 adaptations that increase fitness in mammals (HA-K582I) have severe consequences on viral fitness in waterfowl, and studies in guinea pigs (a favored model for the study of IAV transmission) have also revealed striking differences in the phenotypes associated with H5N1 infections when compared to mice (Gao et al., 2009).

This brings us to the issue of regulatory constraints. Understanding how factors such as virulence, transmissibility, and viral fitness interconnect will require GOF experiments, the use of which has recently been the cause of extensive controversy under the new ''dual-use research of concern (DURC)'' guidelines (Wolinetz, 2012). GOF experiments are (and have always been) a fundamental pillar of scientific inquiry and are essential to the rigorous execution of the scientific method. Indeed, the sensationalization and reactionary blow-back sparked by the original H5N1 transmission studies stem largely from scientific ignorance with regard to how transmission, virulence, and fitness interrelate. Ironically, the only way to address this uncertainty is to move forward with GOF studies that will serve to contextualize how adaptations that mediate mammalian transmissibility affect other properties of the virus (such as the dramatic reduction in virulence observed by the Fouchier group, for example). These studies would serve to demystify the risks and consequences of viral adaptations that lead to mammalian transmissibility and should therefore not be restricted. Certainly, any future hopes for developing a predictive model for pandemic risk assessment will rely on understanding the sum of these properties. It is important to consider that, in the context GOF

experiments related to H5N1 transmission, studies are already being performed in stringently regulated biosafety level 3 (BSL3) facilities by highly skilled individuals. This is a responsible, precautionary approach that ensures the safety of both the scientists performing the studies, and the general public.

Vexing Vaccines

The uncertain principles that determine the pandemic risk of IAV strains are mirrored by similar uncertainties regarding the predictability of vaccine responses designed to prevent IAV infections. A mechanistic understanding of the genetic and environmental factors that account for the heterogeneous nature of individual responses to the same vaccine has long eluded researchers, which in turn has caused vaccine efficacy to suffer. Though this problem is not unique to IAV vaccines, it is additionally complicated by the distinctive qualities of responses elicited by each vaccine formulation (for example: split, inactivated versus live-attenuated). Therefore, the identification of predictive markers of vaccine efficacy is a pressing need. This type of information would not only aide in enhancing the efficacy of current vaccines through more personalized approaches but would also provide a rational basis for the responses required during development of next-generation vaccines.

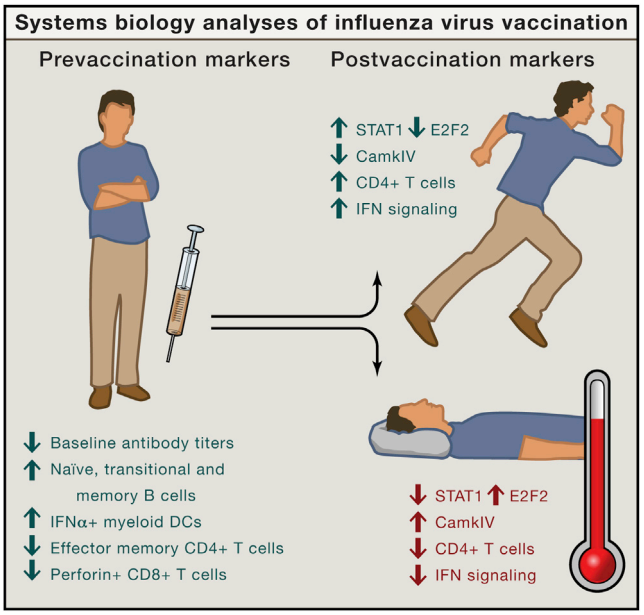

Figure 2. Predictive Factors of Influenza Virus Vaccine Efficacy
Systems biology has facilitated the identification of pre- and postvaccination correlates of efficacy (examples of positive correlates shown in green; negative correlates, red). Elucidation of the mechanistic properties of these markers will allow for personalized approaches that maximize vaccine effectiveness and will guide the development of next-generation vaccines.

Targeted, conventional approaches have had limited success in capturing truly predictive markers that determine the outcome of vaccination. However, recent advances in systems biology offer the opportunity to undertake much more powerful multifactorial analyses. These efforts are beginning to yield tremendous amounts of data regarding the complex orchestration of factors that govern the host response to vaccines, some of which may have predictive value with respect to vaccine efficacy. The seminal study that substantiated the utility of the systems biology approach to evaluate vaccine responses was published in 2009 and explored the response of humans to the highly effective yellow fever virus YF-17D vaccine (Querec et al., 2009). In this issue of *Cell*, Tsang and colleagues (Tsang et al., 2014) describe, for the first time, a set of baseline prevaccination parameters that were found to be predictive of postvaccination antibody responses (Figure 2). The challenge moving forward will be to distill the vast quantities of information obtained through these studies in order to assign biological significance to the observed trends. Indeed, the fusion of systems-level approaches with classical reductionist methods holds the promise of revolutionizing the rational design of vaccines.

One of the earliest systems biology studies of influenza virus vaccine responses focused on transcriptional analyses of peripheral blood mononuclear cells (PBMCs) derived from adult males who received the trivalent inactivated influenza virus vaccine (TIV) (Bucasas et al., 2011). Genes upregulated at early time points postvaccination were largely involved in interferon (IFN) signaling and antigen processing/presentation, whereas genes upregulated later in the response were more likely to be associated with cellular proliferation and protein biosynthesis. Further refinement showed that *STAT1* upregulation was most pronounced 24 hr after vaccination in the high responder group, whereas *E2F2* was downregulated postvaccination, most prominently on day 3 in the high responder group. Remarkably, the postvaccination expression profile of these two genes alone (*STAT1* and *E2F2*) was sufficient to differentiate high and low responders (Bucasas et al., 2011) (Figure 2). These results demonstrated the potential power of systems biology approaches but also highlighted the need for even greater levels of resolution. For which cell types within the PBMC population do these markers primarily apply? What mechanisms are responsible for the observed outcomes? Are these responses specific to TIV, or do they apply more generally to other routes of vaccination?

Elegant work by the Pulendran lab addressed many of these issues by analyzing the immune response to both TIV and live-attenuated influenza virus vaccine (LAIV) in healthy adults over three consecutive seasons (Nakaya et al., 2011). Consistent with previous studies (Sasaki et al., 2007), the authors found that vaccination with TIV elicited the expansion of IgG-secreting plasmablasts more efficiently than administration of LAIV. The PBMC transcriptional signatures associated with each vaccine were also unique. The authors observed marked transcriptional changes in genes related to innate immunity, a trend consistently observed upon transcriptional analyses of influenza virus vaccinees. More recent work has demonstrated that neutrophils and monocytes are primarily responsible for contributing to this early IFN-related gene signature (Obermoser et al., 2013). Upregulation

of genes involved in the type I IFN pathway were especially pronounced for individuals who received LAIV, likely a reflection of its replicative capacity. Interestingly, whereas transcriptional changes associated with B cells correlated positively with antibody titers, T-cell-associated signatures exhibited a negative correlation with the antibody response. Using discriminant analysis via mixed integer profiling (DAMIP), the authors were able to identify and validate a minimal gene signature capable of accurately predicting the antibody responses to TIV. Critically, to confirm the utility of their analyses, the authors selected one of the markers identified in the DAMIP analysis (calcium/calmodulin-dependent protein kinase IV [*CamkIV*]) for functional validation. *CamKIV* expression at day 3 post-TIV vaccination negatively correlated with the magnitude of the antibody response (Figure 2). Vaccination of *CamKIV*$^{-/-}$ mice with TIV recapitulated this effect, inducing higher antibody titers than in control mice (Nakaya et al., 2011).

The biological signatures identified in these and other studies have significantly advanced our understanding of the specific responses elicited by various vaccines. They have also facilitated the identification of postvaccination markers, such as *STAT1*, *E2F2* (Bucasas et al., 2011), *CamkIV* (Nakaya et al., 2011), and CD4+ T cell levels (Nayak et al., 2013; Spensieri et al., 2013), which can accurately predict the magnitude of the antibody response elicited by influenza virus vaccination (Figure 2). However, the ability to predict the outcome of vaccination based on baseline immunological markers has remained elusive. Tsang and colleagues provide a first glimpse of the potential for systems biology to address this problem. In agreement with the work described above (Bucasas et al., 2011; Nakaya et al., 2011; Obermoser et al., 2013), postvaccination PBMC gene expression profiles were characterized by strong enrichment of IFN-related gene expression at day 1 postvaccination, which then shifted to adaptive pathways associated with antibody production by day 7. These results correlated well with changes in cell subset frequencies; CD40+ and CD86+ monocytes and IFNα+ plasmacytoid dendritic cell (DC) populations expanded most on day 1, whereas adaptive cell types, including plasmablasts, B cell subsets, and T cells, were representative of the day 7 response.

Interestingly, high baseline antibody titers were found to be inversely correlated with the postvaccination response. Baseline gene expression and pathway activity were not robust predictors of postvaccination response; however, several pre-vaccination B and T cell subpopulations were found to predict endpoint antibody responses. Naive, transitional, and memory B cell populations were all positively correlated with antibody endpoint titers, whereas effector memory CD4+ T cell and perforin+ CD8+ T cell populations exhibited a negative correlation (Figure 2). Retrospective analyses using these predictive cell populations could then be used to assign functional significance to the gene expression signatures observed from whole PBMCs. It should be noted that the baseline state of antibody titers and cellular composition of PBMCs among individuals is likely to reflect age, at least to some extent. Thus, the strength with which these predictors apply to individuals in different age categories remains to be determined.

Looking into the Future

Experimental assessment of the adaptions required for IAV transmission among mammals and the consequences of these adaptations on other properties of the virus (especially virulence and fitness) promise to provide a more robust framework for assessing the pandemic risk of novel IAV strains (Linster et al., 2014). However, this goal will require a regulatory environment that dissolves the unnecessary barriers that hinder much-needed progress while maintaining a reasonable level of oversight that encourages public trust. In the meantime, the use of systems biology techniques (Tsang et al., 2014) to assemble a more comprehensive picture of the factors that govern the immune response to vaccination should serve as a guide for the design of more efficacious vaccines. Identifying predictive postvaccination markers of efficacy will inform the development of novel adjuvants (Kasturi et al., 2011) that stimulate pathways required for optimal responses, while baseline predictors can be exploited to assess optimal vaccine routes/formulations on an individual basis. Coupled with ambitious ongoing efforts to elicit more broadly protective responses against influenza viruses (Krammer and Palese, 2014), the hope for an effective "universal" influenza virus vaccine may soon be realized.

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REFERENCES

Bucasas, K.L., Franco, L.M., Shaw, C.A., Bray, M.S., Wells, J.M., Niño, D., Arden, N., Quarles, J.M., Couch, R.B., and Belmont, J.W. (2011). Early patterns of gene expression correlate with the humoral immune response to influenza vaccination in humans. J. Infect. Dis. *203*, 921–929.

Gao, Y., Zhang, Y., Shinya, K., Deng, G., Jiang, Y., Li, Z., Guan, Y., Tian, G., Li, Y., Shi, J., et al. (2009). Identification of amino acids in HA and PB2 critical for the transmission of H5N1 avian influenza viruses in a mammalian host. PLoS Pathog. *5*, e1000709.

Girard, M.P., Tam, J.S., Assossou, O.M., and Kieny, M.P. (2010). The 2009 A (H1N1) influenza virus pandemic: A review. Vaccine *28*, 4895–4902.

Herfst, S., Schrauwen, E.J.A., Linster, M., Chutinimitkul, S., de Wit, E., Munster, V.J., Sorrell, E.M., Bestebroer, T.M., Burke, D.F., Smith, D.J., et al. (2012). Airborne transmission of influenza A/H5N1 virus between ferrets. Science *336*, 1534–1541.

Imai, M., Watanabe, T., Hatta, M., Das, S.C., Ozawa, M., Shinya, K., Zhong, G., Hanson, A., Katsura, H., Watanabe, S., et al. (2012). Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. Nature *486*, 420–428.

Julien, J.-P., Lee, P.S., and Wilson, I.A. (2012). Structural insights into key sites of vulnerability on HIV-1 Env and influenza HA. Immunol. Rev. *250*, 180–198.

Kasturi, S.P., Skountzou, I., Albrecht, R.A., Koutsonanos, D., Hua, T., Nakaya, H.I., Ravindran, R., Stewart, S., Alam, M., Kwissa, M., et al. (2011). Programming the magnitude and persistence of antibody responses with innate immunity. Nature *470*, 543–547.

Kimble, J.B., Sorrell, E., Shao, H., Martin, P.L., and Perez, D.R. (2011). Compatibility of H9N2 avian influenza surface genes and 2009 pandemic H1N1 internal genes for transmission in the ferret model. Proc. Natl. Acad. Sci. USA *108*, 12084–12088.

Krammer, F., and Palese, P. (2014). Universal influenza virus vaccines: need for clinical trials. Nat. Immunol. *15*, 3–5.

Linster, M., van Boheemen, S., de Graaf, M., Schrauwen, E.J.A., Lexmond, P., Mänz, B., Bestebroer, T.M., Baumann, J., van Riel, D., Rimmelzwaan, G.F., et al. (2014). Identification, characterization, and natural selection of mutations driving airborne transmission of A/H5N1 virus. Cell *157*, this issue, 329–339.

Nakaya, H.I., Wrammert, J., Lee, E.K., Racioppi, L., Marie-Kunze, S., Haining, W.N., Means, A.R., Kasturi, S.P., Khan, N., Li, G.-M., et al. (2011). Systems biology of vaccination for seasonal influenza in humans. Nat. Immunol. *12*, 786–795.

Nayak, J.L., Fitzgerald, T.F., Richards, K.A., Yang, H., Treanor, J.J., and Sant, A.J. (2013). CD4+ T-cell expansion predicts neutralizing antibody responses to monovalent, inactivated 2009 pandemic influenza A(H1N1) virus subtype H1N1 vaccine. J. Infect. Dis. *207*, 297–305.

Obermoser, G., Presnell, S., Domico, K., Xu, H., Wang, Y., Anguiano, E., Thompson-Snipes, L., Ranganathan, R., Zeitner, B., Bjork, A., et al. (2013). Systems scale interactive exploration reveals quantitative and qualitative differences in response to influenza and pneumococcal vaccines. Immunity *38*, 831–844.

Osterholm, M.T., Kelley, N.S., Sommer, A., and Belongia, E.A. (2012). Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. Lancet Infect. Dis. *12*, 36–44.

Querec, T.D., Akondy, R.S., Lee, E.K., Cao, W., Nakaya, H.I., Teuwen, D., Pirani, A., Gernert, K., Deng, J., Marzolf, B., et al. (2009). Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Nat. Immunol. *10*, 116–125.

Russell, C.A., Fonville, J.M., Brown, A.E.X., Burke, D.F., Smith, D.L., James, S.L., Herfst, S., van Boheemen, S., Linster, M., Schrauwen, E.J., et al. (2012). The potential for respiratory droplet-transmissible A/H5N1 influenza virus to evolve in a mammalian host. Science *336*, 1541–1547.

Sasaki, S., Jaimes, M.C., Holmes, T.H., Dekker, C.L., Mahmood, K., Kemble, G.W., Arvin, A.M., and Greenberg, H.B. (2007). Comparison of the influenza virus-specific effector and memory B-cell responses to immunization of children and adults with live attenuated or inactivated influenza virus vaccines. J. Virol. *81*, 215–228.

Spensieri, F., Borgogni, E., Zedda, L., Bardelli, M., Buricchi, F., Volpini, G., Fragapane, E., Tavarini, S., Finco, O., Rappuoli, R., et al. (2013). Human circulating influenza-CD4+ ICOS1+IL-21+ T cells expand after vaccination, exert helper function, and predict antibody responses. Proc. Natl. Acad. Sci. USA *110*, 14330–14335.

Stern, A.M., and Markel, H. (2005). The history of vaccines and immunization: familiar patterns, new challenges. Health Aff. (Millwood) *24*, 611–621.

To, K.K.W., Chan, J.F.W., Chen, H., Li, L., and Yuen, K.-Y. (2013). The emergence of influenza A H7N9 in human beings 16 years after influenza A H5N1: a tale of two cities. Lancet Infect. Dis. *13*, 809–821.

Tong, S., Zhu, X., Li, Y., Shi, M., Zhang, J., Bourgeois, M., Yang, H., Chen, X., Recuenco, S., Gomez, J., et al. (2013). New world bats harbor diverse influenza A viruses. PLoS Pathog. *9*, e1003657.

Tsang, J.T., Schwartzberg, P.L., Kotliarov, Y., Biancotto, A., Xie, Z., Germain, R.N., Wang, E., Olnes, M.J., Narayanan, M., Golding, H., et al. (2014). Global analyses of human immune variation reveal baseline predictors of postvaccination responses. Cell *157*, this issue, 499–513.

Wolinetz, C.D. (2012). Implementing the new U.S. dual-use policy. Science *336*, 1525–1527.

Zaraket, H., Bridges, O.A., and Russell, C.J. (2013). The pH of activation of the hemagglutinin protein regulates H5N1 influenza virus replication and pathogenesis in mice. J. Virol. *87*, 4826–4834.